Euphytica **115:** 105–113, 2000.

Linkage of random amplified polymorphic DNA markers to downy mildew resistance in cucumber (*Cucumis sativus* L.)

Thomas Horejsi[1], Jack E. Staub[2] & Claude Thomas[3]
[1]*Former Graduate Student, University of Wisconsin Madison; present address: Monsanto, 634 East Lincoln Way, Ames, IA 50010, U.S.A.;* [2]*Author for correspondence: USDA-ARS, Vegetable Crops Unit, Department of Horticulture, 1575 Linden Dr., University of Wisconsin, Madison, WI 53706, U.S.A.;* [3]*USDA-ARS, Vegetable Crop Laboratory, Charleston, SC 29407, U.S.A*

Received 10 June 1999; accepted 21 January 2000

Key words: genetic markers, marker-assisted selection, multiple location analysis, RAPD

Summary

Marker assisted selection (MAS) may improve the efficiency of breeding downy mildew resistant cucumber cultivars. A study was conducted to identify random amplified polymorphic DNA (RAPD) markers linked to the downy mildew resistance gene (*dm*) which would be suitable for MAS. A total of 145 F_3 families from two populations (55 from the WI 1983G × Straight 8 population and 90 from the Zudm1 × Straight 8 population) were evaluated over five locations in North America and Europe. Resistant and susceptible F_3 families were identified and mean family resistance ratings were used to type individual F_2 plants. No evidence for race differences in the pathogen (*Psuedoperonospora cubensis* (Berk. & Curt.) Rostow) between North America and Europe was found. Phenotypic correlations between locations ranged from 0.3 to 0.7. Of the 135 polymorphic RAPD markers identified from 960 primers, five were linked to *dm* – $G14_{800}$, $X15_{1100}$, $AS5_{800}$, $BC519_{1100}$, and $BC526_{1000}$. In the WI 1983G × Straight 8 population, $G14_{800}$ was linked to *dm* at 16.5 cm, $AS5_{800}$ at 32.8 cm, $BC519_{1100}$ at 9.9 cm, and $BC526_{1000}$ at 19.2 cm. In the Zudm1 × Straight 8 population, $G14_{800}$ was linked at 20.9 cm, $X15_{1100}$at 14.8 cm, $AS5_{800}$ at 24.8 cm, and $BC526_{1000}$ at 32.9 cm. Markers $G14_{800}$ and $BC519_{1100}$ were linked in repulsion to the *dm* allele, and $X15_{1100}$, $AS5_{800}$, and $BC526_{1000}$ were linked in coupling phase. These genetic markers may be exploited to develop an efficient MAS strategy for breeding resistant cucumber cultivars.

In cucumber (*Cucumis sativus* L.), many important disease resistance traits are monogenetically inherited (Pierce and Wehner, 1990). The effectiveness of phenotypic selection for disease resistance depends on the pathology of the disease and the inheritance of resistance (Abul-Hayja, 1975). For instance, downy mildew resistance, which is caused by *Psuedoperonospora cubensis* (Berk. & Curt.) Rostow, is controlled by a single gene, *dm* (synonymous with *p*) (Pierce & Wehner, 1990; Fanourakis & Simon, 1987; Van Vliet & Meysing, 1974; Van Vliet & Meysing, 1977). However, phenotypic selection for downy mildew resistance is difficult due to the large influence of environmental factors on the disease's development (Cohen, 1977; Palti & Cohen, 1980), and thus requires the collection of data from multiple replications and years of testing.

Given these difficulties, cucumber breeders would benefit from a more efficient downy mildew resistance breeding procedure such as marker-assisted selection (MAS). With MAS, a breeder bases selection on the marker genotype of a plant instead of (or along with) its phenotype. Linkages between a genetic marker and *dm* may be exploited for MAS (Staub et al., 1996a).

Several documented genetic linkages involving the *dm* gene have been reported. Fanourakis and Simon (1987) found that *dm* is loosely linked to two *cla* (anthracnose resistance, 22.0 cm) and *cp* (compact habit, 38.6 cm). In an F_2 population of a wide cross (*C. sativus* × *C. sativus* var. *hardwickii* (R) Alef.), Kennard et al. (1994) and Dijkhuizen (1994) detected

linkages between *dm* and two flanking RFLP markers, CsC230/*Eco*RI (9.5 cm from *dm*) and CsC593/*Dra*I (17.7 cm from *dm*). Likewise, Meglic and Staub (1996) reported that *dm* was linked to an isozyme allelomorph, *Pgm* (phosphoglucomutase) at 14 cm in a composite map constructed from 28 F_2 families. Because of their large linkage distance from *dm* and the difficulty in assaying marker loci (disease resistance, RFLP, and isozyme), these markers are not particularly good candidates for use in MAS of downy mildew resistance in cucumber. An efficient MAS strategy requires not only markers with tight linkages to *dm*, but also relatively easy to assay.

Random amplified polymorphic DNA (RAPD) markers are relatively simple, inexpensive and are amenable to the high through-put analysis (Williams et al., 1990). RAPD markers have been successful in cucumber genetic mapping; a RAPD linkage map has been constructed using 77 RAPD markers and three morphological traits [sex expression (*F*), determinate habit (*de*), and little leaf (*l*)] (Serquen et al., 1997). If RAPD markers linked to *dm* could be identified, they may be used for MAS in applied cucumber breeding programs. However, the effectiveness of MAS not only depends upon the genetic marker's ease of assaying, but also on the level of resistance provided by the *dm* gene. If isolates of *P. cubensis* overcome the genetic resistance provided by *dm* or if different races of pathogen exist, then MAS based on linked markers would not be effective. Therefore, a study was designed to: 1) examine downy mildew resistance data for evidence of race-specific pathogen variability, and 2) identify RAPD markers linked to *dm* in cucumber. Data from this study may provide for the establishment of more efficient downy mildew resistant breeding strategies in cucumber.

Materials and methods

Development of experimental populations

Two F_2 populations were created to detect linkages between *dm* and RAPD markers. The evaluation and selection of potential mapping parents was based on plant phenotype and consistent responses to the pathogen challenge (i.e. distinct resistance or susceptibility). To increase the likelihood that the RAPD markers used in this study are useful in applied breeding populations, we felt it was necessary to restrict our choice of parents to adapted germplasm. When genetic mapping is conducted in populations from a cultivated × wild species cross, such as *Cucumis sativus* var. *sativus* × *Cucumis sativus* var. *hardwickii* (cultivated cucumber × wild cucumber), the markers are generally not polymorphic among adapted germplasm (Kennard et al., 1994; Dijkhuizen, 1994). Thus, parental selection for F_2 population development involved the screening of 40 adapted lines and cultivars for downy mildew resistance.

One susceptible, 'Straight 8,' and two resistant, 'WI 1983G' and Zudm1, parents were chosen based on their performance during the initial seedling screening tests (Abul-Hayja, 1975). Straight 8 is an old, indeterminate, monoecious, white-spined, open-pollinated American slicing cucumber variety developed by Ferry-Morse Seed Company in 1935 (Tapley et al., 1937). The inbred line WI 1983G was developed by the U.S. Department of Agriculture and the University of Wisconsin-Madison in 1986 (Peterson et al., 1986). It is a multiple disease resistant, gynoecious, white-spined, non-bitter, indeterminate American pickling line. The source of the resistance allele (*dm*) in WI 1983G was derived from 'GY14' and traces back to PI 197087. This is the same allele that was mapped by Kennard et al. (1994) and Fanourakis & Simon (1987). The inbred line Zudm1 was developed by Zaadunie, Seeds B.V. (formerly S&G Seeds and presently Novartis, Bleiswijk, The Netherlands), and is an indeterminate, gynoecious, parthenocarpic, white-spined Chinese long type cucumber (origin of the *dm* resistance gene is not known).

Downy mildew evaluations

Field and greenhouse evaluations were conducted in the USA and Europe. For linkage studies, the F_3 families from two populations (WI 1983G × Straight 8 and Zudm1 × Straight 8) were tested for resistance to *P. cubensis* using a randomized complete block design with two to four replications at five locations. The mean level of resistance of each F_3 family was used to infer the resistance level of individual F_2 plants. Sixty-two F_3 families from the WI 1983G × Straight 8 population were evaluated over four locations with two or three replications at a location (Table 1). Due to poor germination of seven F_3 families, only 55 were analyzed in this population. For the Zudm1 × Straight 8 population, 90 F_3 families were evaluated over five locations having two to four replications at a location. Plant disease reactions were assessed by the appearance of cotyledon lesions and were scored on a

Table 1. Locations of downy mildew reaction evaluation in two cucumber populations

Location[a]	Reps[b]	Plants /plot[c]	Test type[d]	Inoculation method[e]	Evaluation date[f]
WI 1983G × Straight 8 Population					
Madison, Wisconsin	3	10	greenhouse	artificial	6/1/95–8/8/95
Almeria, Spain	2	5	greenhouse	artificial	11/24/94–1/6/95
Charleston, South Carolina	2	9	field	artificial	8/31/94–10/7/94
Haelen, The Netherlands	3	10	field	natural	6/22/95–8/23/95
Zudm1 × Straight 8 Population					
Madison, Wisconsin	4	10	greenhouse	artificial	8/11/95–10/7/95
Almeria, Spain	2	10	greenhouse	artificial	10/25/95 11/27/95
Bleiswijk, The Netherlands	2	10	greenhouse	artificial	10/22/95–11/4/95
Charleston, South Carolina	2	10	field	artificial	8/14/95–10/11/95
Haelen, The Netherlands	2	10	field	natural	6/24/96–8/26/96

[a] University of Wisconsin, Madison, Wisconsin; U.S. Department of Agriculture Vegetable Laboratory, Charleston, South Carolina; S & g seeds research station, Almeria, Spain; Nunhems research station, Haelen, The Netherlands and De Ruiter research station, Bleiswijk, The Netherlands.
[b] Number of replications.
[c] Plants per plot.
[d] Evaluation site.
[e] Natural signifies no inoculum applied and artificial signifies external inoculum applied.
[f] Dates of evaluation (month/day/year).

0 to 9 scale using a visual rating system based on the appearance of lesions where:

0 = no symptoms,
1 = faint spot,
2 = necrotic spot, no chlorosis,
3 = pale yellow spot,
4 = necrotic spot with pale yellow border,
5 = yellow spot,
6 = necrotic spot with yellow border,
7 = bright yellow spot,
8 = bright yellow spot with slightly necrotic center, and
9 = bright yellow spot with necrotic center.

Greenhouse seedling tests were conducted at the University of Wisconsin-Madison as described by Abul-Hayja (1975). The seeds were sown into wooden flats (52 × 36 × 7 cm) filled with steam-sterilized vermiculite. Each flat contained both a positive (susceptible line SMR-18) and a negative (resistant line Gy14) control. In those cases where SMR18 showed no symptoms or variable symptoms the data from that replication was thrown out. The flats were placed on a heated germination bench for 3 to 4 days. A fully expanded cotyledon from each seedling was inoculated with a single drop (approximately 0.01 to 0.03 ml) of inoculum, containing 12×10^4 sporangia per milliliter. In each case, the second cotyledon was punctured with a pasteur pipette to mark the inoculated seedlings such that late germinating seedlings were not included in the evaluation. Flats were placed inside plastic boxes and were incubated at 20 °C with approximately 100% relative humidity in darkness for two days. Flats were then placed in a greenhouse (24 ° to 30 °C) in a 16 hour photoperiod, and disease symptoms were scored seven to eight days after inoculation.

In Charleston, South Carolina, field evaluations were performed using two replications of F_3 families from each population (Table 1). Uniform one to two-week old seedlings were transplanted to single rows where the plants were spaced 0.6 m apart on 1.5 m row centers. To hasten infection and help remove border effects, spreader rows of the susceptible line SMR18 were planted in every third row, such that each plot was bordered by susceptible plants. The field was misted with inoculum containing 25×10^3 sporangia per milliliter approximately four weeks after transplanting. The plants were then rated six weeks after transplantation.

Greenhouse evaluations in Almeria, Spain were performed using two replications of either five (WI 1983G × Straight 8 population) or ten (Zudm1 × Straight 8 population) plants per plot (Table 1). The plants were inoculated approximately three weeks

after planting with a pathogen culture isolated from local commercial fields. The plants were rated for disease reaction 11 to 16 days after inoculation.

Field evaluations at the Haelen, The Netherlands location involved examination of three (WI 1983G × Straight 8 population) or two (Zudm1 × Straight 8 population) replications (Table 1). The seeds were germinated in a greenhouse and were transplanted into the field after about 10 days (at two-leaf stage) where they were challenged by severe natural infection (personal communication, Jos Suelmann, Nunhems Zaden BV, Healen, The Netherlands, 1995 and 1996). The row plots had 20 cm spacings between plants and 2 m between rows. The plants were rated for pathogen response five to eight weeks after transplantation.

In Bleiswijk, greenhouse evaluations were performed only on the Zudm1 × Straight 8 population (Table 1). This evaluation involved two replications of ten plants of each family. The plants were inoculated (8.5×10^3 sporangia per milliliter) five weeks after planting and rated for disease reaction after an additional week.

The mean level of resistance of each F_3 family was used to infer the resistance level of individual parent (F_2) plants. Visual observation of the downy mildew score distribution (e.g. histograms), was used to identify transition points between classes (resistant, intermediate and susceptible) according to the single gene model of resistance (Van Vliet & Meysing, 1974). Genotypes of individual F_2 plants were assigned into three genotypic classes based on their corresponding F_3 family performance (*dm/dm* for resistant, *Dm/dm* for intermediate and *Dm/Dm* for susceptible).

Analyses of variance (ANOVA) were performed using plot mean downy mildew scores for each location and combined over locations for each population (Gomez & Gomez, 1984). All effects were considered random. Genetic variance (σ_g^2), location variance (σ_l^2), replication variance (σ_r^2), genotype by location variance (σ_{gl}^2), and error variance (σ^2) components were calculated from the partitioned sources of variation (Nyquist, 1991; Hallauer & Miranda, 1988). Broad-sense heritabilities (H^2) on a single-plant basis were estimated as: $H^2 = \sigma_g^2 / \sigma_{ph}^2$; where $\sigma_{ph}^2 = \sigma^2 + \sigma_{gl}^2 + \sigma_l^2 + \sigma_g^2$. Phenotypic correlations among locations were calculated by Pearson correlation using SAS software (SAS institute Inc., Cary, NC).

RAPD marker evaluation

The RAPD analysis was similar to that of Williams et al. (1990) with modifications for cucumber (Staub et al., 1996b). DNA was extracted from young leaf tissue of F_2 plants using a CTAB extraction procedure (Staub et al., 1996b; Maniatis et al., 1982). The DNA was then quantified on a Hoefer TKO 100 mini-fluorometer (Hoefer Scientific Instruments, San Francisco, California) following the manufacturer's protocol. This instrument calculates the DNA concentration by light absorbance comparisons between the unknown and standard DNA (calf thymus DNA) solutions.

All polymerase chain reaction (PCR) solutions were purchased from Promega (Madison, Wisconsin). Each PCR had a volume of 15 μl and contained 3.0 mM $MgCl_2$, 0.2 mM dNTPs (0.05 mM of each: dATP, dGTP, dTTP and dCTP), 15 ng DNA, 0.3 μM primer, commercial polymerase buffer and one unit Taq DNA polymerase. The 10-mer primers A1 to AX20 were purchased from Operon Technologies (Alameda, California), and the primers BC200 to BC699 were obtained from the University of British Columbia (Vancouver, BC, Canada). PCRs were conducted with the Perkin Elmer GeneAmp PCR System 9600 (Norwalk, Connecticut) thermocycler using the following cycling profile: 94 °C/ 4 min; 3 cycles of 94 °C/ 15 sec, 35 °C/ 15 sec, 59 sec ramp to 72 °C/ 75 sec; 40 cycles of 94 °C/ 15 sec, 40 °C/ 15 sec, 59 sec ramp to 72 °C/ 75 sec; 72 °C/ 7 min and indefinite soak at 4 °C.

After completion of the PCR, 3 μl of loading dye (0.25% bromophenol blue, 0.25% xylene cyanoll FF, 15% Ficol) was added to each reaction tube. The samples were electrophoresed in 1.6% agarose gels (20 × 25 cm) containing 0.5 mg/ml ethidium bromide in TAE buffer [4.84% tris (trishydroxymethylaminomethane), 1.14% acetic acid, 0.375% EDTA] for three hours at approximately 100 volts. The gels were immediately photographed using the Eagle Eye still video system (Stratagene, LaJolla, California).

Repeatable banding patterns were scored as presence (+) or absence (–) of a DNA band. A marker was considered repeatable if PCR yielded a consistent result in all of four (or more) replications. DNA fragment sizes were estimated by comparison of migration distance to that of *Hind*III + *Eco*RI digested lambda-phage DNA. Each band is identified by the RAPD primer and the PCR product fragment size. For example, $G14_{800}$ designates a 800 base-pair band ampli-

fied from the G14 primer and $BC526_{1000}$ designates a 1000 base-pair band from the BC526 primer.

Bulk segregant analyses (Michelmore et al., 1991) were employed for the identification of RAPD markers potentially linked to *dm*. In the WI 1983G × Straight 8 population, the bulks consisted of DNA from four resistant F_2 plants and four susceptible F_2 plants as defined by F_3 family phenotypes. In the Zudm1 × Straight 8 population a similar bulking procedure was employed with DNA from five resistant and five susceptible F_2 plants. Michelmore et al., (1991) derived the equation: $2[1–(0.25)^N][0.25]^N$ for calculating the probability of a false association (marker unlinked to target gene), where N = the number of individuals in each bulk. While with four plants in each bulk the probability of observing a false association is 0.008, with five plants the probability decreases to 0.002.

Once potential linkages were identified, PCRs using appropriate RAPD primers were conducted on DNA from individual F_2 plants to collect data for genetic linkage analysis. Genetic linkages were estimated using the Mapmaker 2.0 computer program (Lander et al., 1987) and Linkage-1 for DOS (Suiter et al., 1983). Mapmaker uses a likelihood algorithm to calculate the most likely map and was set at a minimum LOD of 3.0 and a maximum recombination frequency (theta) of 0.40. In contrast, Linkage-1 estimates recombination by two point analysis based on a chi-square goodness-of-fit algorithm. Recombination frequencies were transformed into centiMorgans (cM) using the Kosambi mapping function.

Results and discussion

Downy mildew resistance data

F_3 families of WI 1983G × Straight 8 and Zudm1 × Straight 8 populations were evaluated for level of downy mildew resistance. The large error variances indicated that downy mildew resistance is difficult to characterize (data not presented; Horejsi, 1998). Large experiment-wide coefficient of variation (CV) values were detected, 19.6 and 28.1% for the WI 1983G × Straight 8 for Zudm1 × Straight 8 populations, respectively. Broad sense heritability (per plant basis) estimates for downy mildew resistance were relatively low (H^2 = 8 and 17% for the WI 1983G × Straight 8 and the Zudm1 × Straight 8 populations, respectively). The low heritability estimates may be a reflection of large plant-to-plant variability, which confirms the need for replicated testing for the downy mildew resistance trait.

Table 2. Phenotypic correlation coefficients of downy mildew resistance scores of F_3 families from the WI 1983G × Straight 8 cucumber population at three locations

Location	Spain	Wisconsin	The Netherlands
South Carolina	0.54**	0.48**	0.69**
Spain		0.48**	0.55**
Wisconsin			0.61**

** Significant at the 0.01 probability level.

Table 3. Phenotypic correlation coefficients of downy mildew resistance scores of F_3 families from the Zudm1 × Straight 8 cucumber population at four locations

Location	South Carolina	Haelen, The Netherlands	Spain	Bleiswijk, Netherlands
Wisconsin	0.48**	0.56**	0.26*	0.63**
South Carolina		0.36**	ns	0.33**
Haelen			0.32**	0.47**
Spain				0.25*

**, * Significant at the 0.01 and 0.05 probability level, respectively.
ns = not significant.

Significant variation was detected among locations (data not presented; Horejsi, 1998). However, the most resistant families in one location tended to be most resistant in the other locations such that the rankings of the F_3 families stayed relatively constant over locations. Correlation coefficients of downy mildew scores between locations were highly significant ($p < 0.01$) in many cases (Tables 2 and 3). Correlation coefficients for the WI 1983G × Straight 8 population ranged from 0.48 to 0.69. Although the correlation between South Carolina and Spain was not significant in the Zudm1 × Straight 8 population, correlations between other locations ranged from 0.25 to 0.63. Thus, each downy mildew test location provided similar F_3 family disease reaction data and the data was pooled over locations.

European test locations tended to have higher downy mildew scores (mean rating 5.9 ± 2.1) than the North American locations (5.1 ± 0.5) (data not presented, Horejsi, 1998). It might be hypothesized that each continent harbors different races of the pathogen. Since the same families were evaluated, downy mildew score differences between locations may have been due to environmental and/or pathogen factors. If race-specific pathogen differences caused the ob-

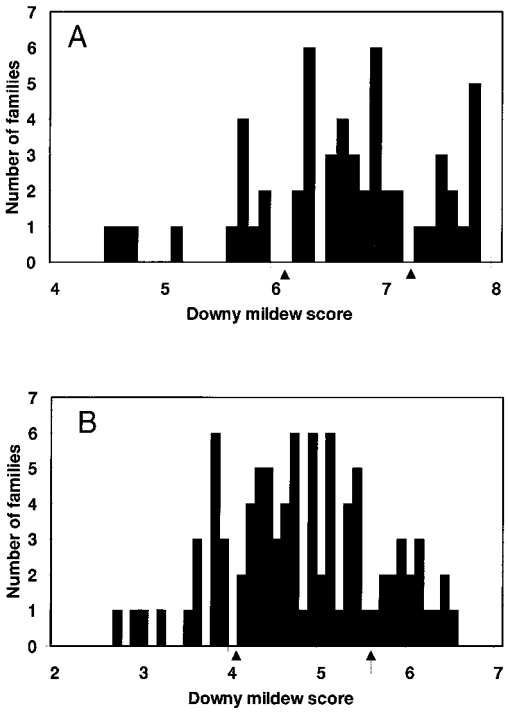

Figure 1. Genetic linkages in cucumber consisting of the downy mildew resistance gene (*dm*) and RAPD markers constructed from: the WI 1983G × Straight 8 derived population, panel A and the Zudm1 × Straight 8 derived population, panel B.

served location variation, changes in the ranking of genotypes would be predicted. Such changes were not evident, and thus the data provide no evidence to suggest that races of *P. cubensis* in North America and Europe are different.

Downy mildew in cucumber can be devastating to European growers (Van Vliet & Meysing, 1974). European growing environments may be so conducive to disease development that the *dm* allele does not provide for adequate control of disease damage. Where downy mildew reaches epidemic proportions, the level of resistance provided by the *dm* allele appears to be unsatisfactory. Thus, breeders may be forced to search for other more resilient resistance genes (new locus) or alleles (same locus as *dm*).

Even with the severe disease symptoms from the European evaluation, individual F_2 plants could be grouped into three genotype classes based on discontinuities in the distribution of corresponding F_3 family downy mildew scores (Figure 1, Horejsi, 1998). In the WI 1983G × Straight 8 population, the scores of resistant (*dm/dm*) families ranged from 4.5 to 5.9, intermediate (*Dm/dm*) families ranged from 6.2 to 7.1, and susceptible (*Dm/Dm*) families ranged from 7.3 to 7.8. Due to difference in test years, in the Zudm1 × Straight 8 population, the scores of resistant (*dm/dm*) families ranged from 2.7 to 3.9, intermediate (*Dm/dm*) families ranged from 4.1 to 5.4, and susceptible (*Dm/Dm*) families ranged from 5.5 to 6.5.

Of the 55 F_2 progeny evaluated in the WI 1983G × Straight 8 population, 12 were resistant, 30 were intermediate and 13 were susceptible. A chi-square test for fit of a 1: 2: 1 ratio (single codominant gene) indicates that the observed segregation was not significantly different from the expected ($\chi^2 = 0.49$, $p > 0.75$). Of the 90 F_2's evaluated in the Zudm1 Straight 8 population, 17 were resistant, 54 were intermediate and 19 were susceptible. Likewise, the genotypic distribution for disease reaction in this population was not significantly different from the expected ($\chi^2 = 3.69$, $p > 0.10$).

Even though the progeny in these populations segregated as predicted, it is possible that genotype classification errors did occur. Errors in classification would bias estimates of genetic recombination and lead to either the over- or under-estimation of map distance (Horejsi, 1998). Linkage estimates are based on classifying gametes as either parental or recombinant. Thus, misclassification of parental as recombinant gametes increases the linkage estimate. In contrast, misclassification of recombinant gametes as parental gametes decreases the linkage estimate. In cases where a true linkage exists, parental gametes are more frequent than recombinant gametes. If one assumes these errors are random, more parental to recombinant misclassifications would be expected. Since the potential bias of genotype misclassification favors an increase in linkage estimate, the values reported here may be overestimated.

Linkage analysis

A total of 960 primers were used in the preliminary screening of the parents (WI 1983G, Zudm1 and Straight 8) to identify potential RAPD marker polymorphisms. Of approximately 6,500 candidate bands, 135 (2.0%) yielded repeatable, reliable polymorphisms. Only 62 (1.0%) of these bands were polymorphic between WI 1983G and Straight 8, and 103 (1.6%) were polymorphic between Zudm1 and Straight 8.

Bulked segregant analyses were employed as preliminary tests of linkage between polymorphic RAPD markers and *dm*. Four polymorphisms ($X15_{1100}$,

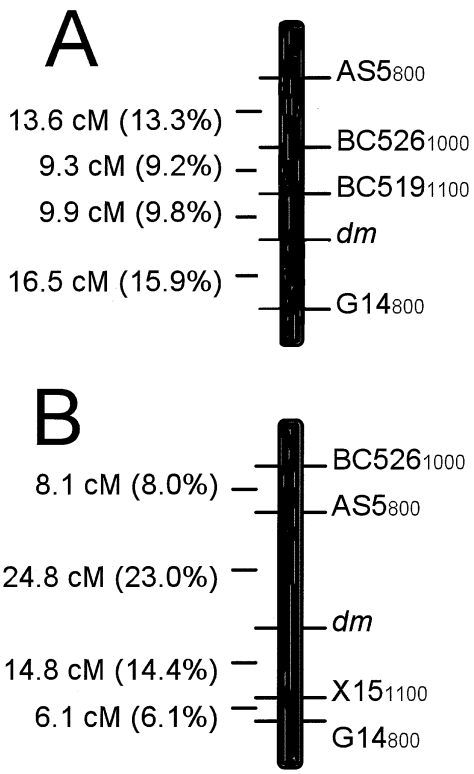

Figure 2. Histograms of downy mildew ratings of F_3 families in the WI 1983G × Straight 8 derived population, panel A and the Zudm1 × Straight 8 derived population, panel B. Arrows indicate break-points between resistant, intermediate and susceptible classes. $G14_{800}$ and $BC519_{1100}$ were linked in repulsion phase to the *dm* allele, and $X15_{1100}$, ASS_{800} and $BC526_{1000}$ were linked in coupling phase.

$G14_{800}$, $BC526_{1000}$ and $BC519_{1100}$) were identified in the WI 1983G × Straight 8 population and three ($X15_{1100}$, $G14_{800}$ and $BC526_{1000}$) were detected in the Zudm1 × Straight 8 population with potential linkage to the *dm* locus. Analysis of individual F_2 plants (55 in the WI 1983G × Straight 8 population and 90 in the Zudm1 × Straight 8 population) revealed that five markers were linked to the *dm* locus ($G14_{800}$, $X15_{1100}$, $AS5_{800}$, $BC519_{1100}$ and $BC526_{1000}$) (Figure 2). A series of 16 widely dispersed mapped RAPD markers (Serquen et al., 1997) were also scored on these F_2 plants as reference point markers. Of these only $AS5_{800}$ showed a linkage to *dm*. In the WI 1983G × Straight 8 population (panel A), $G14_{800}$ was linked to *dm* at 16.5 cm, $AS5_{800}$ at 32.8 cm, $BC519_{1100}$ at 9.9 cm, and $BC526_{1000}$ at 19.2 cm. In contrast, in the Zudm1 × Straight 8 population (panel B), $G14_{800}$ was linked at 20.9 cm, $X15_{1100}$at 14.8 cm, $AS5_{800}$ at 24.8 cm, and $BC526_{1000}$ at 32.9 cm. Markers $G14_{800}$ and $BC519_{1100}$ were linked in repulsion to the *dm* allele, and $X15_{1100}$, $AS5_{800}$, and $BC526_{1000}$ were linked in coupling phase.

Although the most likely maps derived from the Mapmaker program for each population were in general agreement, they differed in two distinct ways. First, marker $X15_{1100}$ was linked (14.8 cm) in the Zudm1 × Straight 8 population, but no such linkage was detected in the WI 1983G × Straight 8 population (Figure 2, panels A and B). This inconsistency may be partially explained by the small number of F_2 progeny used to construct the WI 1983G × Straight 8 map. Second, marker loci order varied such that in the WI 1983G × Straight 8 population, $BC526_{1000}$ was placed between *dm* and $AS5_{800}$, but in the Zudm1 × Straight 8 population, $AS5_{800}$ mapped between *dm* and $BC526_{1000}$. This marker position reversal may be partially explained by the fact that these markers were in repulsion linkage phase. Since RAPD markers are dominant, detection of recombinant types between markers in repulsion phase is difficult.

The recombination fraction estimates of the Linkage-1 computer program were in general agreement with that of Mapmaker (data not presented; Horejsi, 1998). However, Linkage-1 detected no recombination between $BC526_{1000}$ and $BC519_{1100}$ in the WI 1983G × Straight 8 map. Similarly, no recombination was detected between $X15_{1100}$ and $G14_{800}$ in the Zudm1 × Straight 8 map. These discrepancies in recombination estimates results in lowered confidence in the order of these markers, and is probably a reflection of the relatively small population size used (55 for the WI 1983G × Straight 8 population and 90 for the Zudm1 × Straight 8 population) for linkage analysis. Standard errors of linkage estimates ranged between 3.4 (linkages *dm* – $G14_{800}$, and *dm* – $X15_{1100}$) to 13.2 (linkages $AS5_{800}$ - $BC526_{1000}$, and $AS5_{800}$ – $BC519_{1100}$). The large standard error between linkage pair $AS5_{800}$ and $BC526_{1000}$ [13.4% ± 13.2 (Zudm–1 × Straight 8 map) and 8.3% ± 10.5 (WI 1983G × Straight 8 map)] indicates a lowered level of confidence. This may partially explain the discrepancy between the WI 1983G × Straight 8 derived and the Zudm1 × Straight 8 derived maps of placement of $AS5_{800}$ and $BC526_{1000}$ loci.

The marker loci $AS5_{800}$ and $BC526_{1000}$ were previously mapped to linkage group B in cucumber (Serquen et al., 1997). Therefore, *dm* should also reside on this linkage group. Linkage group B was the longest (199 cm) of nine groups identified, and contains 23 of the 77 (30%) mapped RAPD marker loci with a

mean linkage distance of 7.7 cm. The abundance of RAPD marker loci on the same linkage group as *dm* increases the likelihood for successful use of marker loci for MAS for downy mildew resistance in cucumber. Also, RAPD markers may be converted into other types of markers, such as SCAR markers, (Horejsi et al., 1999) which may be more amenable to the high throughput analysis necessary for applying MAS in a large breeding program.

The RAPD markers linked to *dm* identified in this study might be used in MAS to increase recovery of downy mildew resistant genotypes from conventional screening tests. Selection for downy mildew resistance could be accomplished by MAS using the RAPD markers identified here ($G14_{800}$, $X15_{1100}$, $AS5_{800}$, $BC519_{1100}$, or $BC526_{1000}$). Since selection is typically for homogeneously homozygous resistant (*dm/dm*) genotypes, only 25% of the F_3 families derived from a resistant (*dm/dm*) susceptible (*Dm/Dm*) mating are selected. Since most (75%) of the F_3 families will be discarded, the population from which selection for additional traits can practiced will be relatively small. However, with MAS, a smaller portion of families ($< 75\%$) will be discarded during the conventional screening tests, leaving more families ($> 25\%$) available for selection of additional traits. Therefore, MAS would improve the overall breeding efficiency.

MAS efficiency depends on recombination fraction and linkage phase of the marker to the target gene (*dm*) (Staub et al., 1996a). MAS is more efficient when using flanking rather than single markers, and repulsion phase markers are more effective than coupling phase markers for recovery of target traits in MAS (Horejsi, 1998; Haley et al., 1994). Thus, MAS for downy mildew resistance in cucumber is theoretically optimized when flanking markers are in repulsion phase linkage. The flanking markers $G14_{800}$ and $BC519_{1100}$ reported here are linked in repulsion (Figure 2, panel A) and have potential for MAS. Testing of a MAS strategy for downy mildew resistance in cucumber using linked markers will be required to determine its feasibility and method of deployment for breeding.

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